Levensduur van messenger-RNA in rattepancreas

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INLEIDING

De synthese van enzymen verloopt in alle organismen gereguleerd, doordat laagmoleculaire verbindingen specifiek - als effectoren - de synthese van bepaalde enzymen kunnen versnellen of vertragen. Over de regulering der eiwitsynthese in bacteriën is al vrij veel bekend; over die in dieren, bijvoorbeeld gewervelde dieren, aanzienlijk minder.

Zoals later zal worden uiteengezet, kan regulering van de synthese van specifieke enzymen plaatshebben zowel bij de synthese van messenger-RNA (mRNA) als bij het gebruik ervan bij de enzymsynthese zelf. Voor een inzicht in de regulering is derhalve kennis van de levensduur van hun mRNA essentieel.

Bij bacteriën was men er reeds in geslaagd deze te bepalen[1]; daarbij waren zeer korte halfwaardetijden gevonden. Over de levensduur van mRNA in dierlijke systemen van gespecialiseerde cellen was bij het begin van dit onderzoek weinig bekend. Daarom werd besloten de levensduur van de mRNA van enkele pancreasenzymen te onderzoeken.

Daar de hoeveelheid van deze mRNA niet direct bepaald kan worden, werd dit indirect gedaan door de synthese van de betrokken enzymen te bepalen na stopzetting van de RNA-synthese met het antibioticum actinomycine D. In principe kan uit het verloop van de snelheid van de enzymsynthese na toediening van actinomycine D de halfwaardetijd van de mRNA voor het betrokken enzym worden bepaald.

In hoofdstuk I wordt een overzicht gegeven van enzymreguleringen in het algemeen. In hoofdstuk II volgt een uiteenzetting van de overwegingen die tot de keuze van de rattepancreas als studieobject hebben geleid en een overzicht van hetgeen reeds bekend is over de regulering van de synthese der pancreasenzymen. Vervolgens wordt in hoofdstuk III dieper ingegaan op methoden ter bepaling van de levensduur van mRNA en worden overwegingen gegeven betreffende het toepassen van actinomycine D bij ons onderzoek. De laatste twee hoofdstukken tenslotte beschrijven de gebruikte methoden en de resultaten van het eigen onderzoek, waarin de invloed van actinomycine D op de RNA-synthese in vivo en de eiwit- en enzymsynthese in vitro werd nagegaan. Het laatste hoofdstuk bevat tevens een discussie van de verkregen resultaten.

SUMMARY

In the exocrine pancreas few types of enzyme proteins are produced and secreted in great amounts. The relative amount of each of these enzymes is diet-dependent. The synthesis of these proteins is therefore apparently subjected to regulatory control.

As this control might operate at the level of messenger RNA synthesis, I investigated the life-time of messenger RNA's for chymotrypsinogen, trypsinogen, amylase and ribonuclease. For this purpose, RNA synthesis was blocked by actinomycin D treatment in vivo and the synthesis of these proteins was measured in tissue pieces in vitro at different times after cessation of RNA synthesis.

RNA synthesis was measured by determining the degree of incorporation of labelled phosphate into total pancreatic RNA and by comparing its value with the degree of incorporation into the nucleotide pool. This was necessary as we found that the incorporation into the nucleotide pool, which normally shows some variability, may be drastically lowered by actinomycin D and then shows widely diverging values in different experiments. By comparing the two measurements we could avoid a wrong interpretation of lowered labelling of RNA.

As rat pancreas contains large amounts of ribonuclease, a special isolation procedure had to be found in order to obtain intact RNA. The following method gave good results: Pancreas tissue was frozen in liquid nitrogen and lyophilized. The dry powder was treated with hot phenol in the presence of sodium dodecylsulphate and bentonite - to inhibit ribonuclease action - and saline was added to extract the RNA. Undegraded RNA was obtained in good yield in this way.

In the nucleotide pool, the radioactivity of the α-phosphate (next to ribose) was determined by hydrolyzing the pyrophosphate linkages in saturated $Ca(OH)_2$. Charcoal adsorption separated the nucleotides from other components and specific activity of the α-phosphate was determined after combustion of the charcoal-adsorbed mixture.

Different doses of actinomycin D were tried in order to obtain full inhibition of RNA synthesis, while at the same time the animals should survive the treatment. A dose of 1 mg per kg rat, injected intraperitoneally every six hours, appeared to lead to maximal inhibition of RNA synthesis. By sucrose gradient analysis we could show that the residual incorporation is not due to RNA synthesis but is caused by

exchange reactions in the endgroup of transfer RNA.

The enzyme synthesis measured in vitro was shown by several criteria to be true protein synthesis: amino acids were indispensable and the amount of total protein increased on incubation. Up to 18 hours after complete inhibition of RNA synthesis, synthesis of chymotrypsinogen, trypsinogen, amylase and ribonuclease was normal in spite of the bad condition of the animals. The half-life of the messenger RNA's for these enzymes is therefore certainly more than 18 hours.

When RNA synthesis had been blocked for more than 18 hours enzyme synthesis decreased. Whether this decrease was due to partial exhaustion of the messenger RNA's could not be decided in view of the extremely bad condition of the animals and the known side-effects of actinomycin D.

Therefore we may only conclude that the messenger RNA of most of the digestive enzymes from rat pancreas have a half-life exceeding 18 hours. It is impossible by present methods to determine this value more accurately, but it is quite clear that in this specialized tissue messenger half-life for some export-enzymes is long and constitutes an example of the stability of messenger RNA in specialized cells.